

Amendments to the Specification:

Please replace the paragraph beginning at page 6, line 12, with the following:

--Figure 2 shows the amino acid sequence of p110^{RB} (SEQ ID NO:8).--

Please replace the paragraph beginning at page 6, line 13, with the following:

--Figure 3 shows a DNA sequence encoding a retinoblastoma tumor suppressor protein (SEQ ID NOS:7 and 8).--

Please replace the paragraph beginning at page 8, line 8, with the following:

--Figure 9 is expression of rAd/p53 RNA in established tumors. H69 (SCLC) cells were injected subcutaneously into nude mice and allowed to develop tumors for 32 days until reaching a size of approximately 25-50 mm³. Mice were randomized and injected peritumorally with 2 x 10⁹ pfu of either control A/C/ β -gal or A/C/53 virus. Tumors were excised 2 and 7 days post injection, and polyA RNA was prepared from each tumor sample. RT-PCR was carried out using equal RNA concentrations and primers specific for recombinant p53 message. PCR amplification was for 30 cycles at 94°C 1 min., 55°C 1.5 min., 72°C 2 min., and a 10 min., 72°C final extension period in an Omnigen thermocycler (Hybaid). The PCR primers used were a 5' Tripartite Leader cDNA (5' - CGCCACCGAGGGACCTGAGCGAGTC-3'; SEQ ID NO:1) and a 3' p53 primer (5' - TTCTGGGAAGGGACAGAAGA-3'; SEQ ID NO:2). Lanes 1, 2, 4, and 5 are p53 treated samples excised at day 2 or 7 as indicated. Lanes 3 and 6 are from β -gal treated tumors. Lanes 7, 8, and 9 are replicates of lanes 4, 5, and 6 respectively, amplified with actin primers to verify equal loading. Lane 10 is a positive control using a tripartite/p53 containing plasmid.--

Please replace the paragraph beginning at page 17, line 16, with the following:

--Another example of a tumor suppressor gene is retinoblastoma (RB). The complete RB cDNA nucleotide sequences and predicted amino acid sequences of the resulting RB protein (designated p110^{RB}) are shown in Lee *et al.* (1987) and in Figure 3 (SEQ ID NOS:7 and 8). Also useful to express retinoblastoma tumor suppressor protein is a DNA molecule encoding the amino acid sequence shown in Figure 2 (SEQ ID NO:8) or having the DNA sequence shown in Figure 3 (SEQ ID NOS: 7 and 8). A truncated version of p110^{RB}, called p56^{RB}, also is useful. For the sequence of p56^{RB}, see Huang *et al.* (1991). Additional tumor suppressor genes can be used in the vectors of this invention. For illustration purposes only, these can be p16 protein (Kamb *et al.* (1994)), p21 protein, Wilm's tumor WT1 protein, mitosin, h-NUC, or colon carcinoma DCC protein. Mitosin is described in X. Zhu and W-H Lee, U.S. Application Serial No. 08/141,239, filed October 22, 1993, and a subsequent continuation-in-part by the same inventors, attorney docket number P-CJ 1191, filed October 24, 1994, both of which are herein incorporated by reference. Similarly, h-NUC is described by W-H Lee and P-L Chen, U.S. Application Serial No. 08/170,586, filed December 20, 1993, herein incorporated by reference.--

Please replace the paragraph beginning at page 19, line 4, with the following:

--An example of a vector of this invention is a recombinant adenovirus expression vector having a foreign gene coding for p53 protein or an active fragment thereof is provided by this invention. The ~~coding~~ amino acid sequence of the p53 ~~gene~~ polypeptide is set forth below in ~~Table I~~ Table 1 (SEQ ID NO:9).--

Please replace the paragraph (TABLE 1) beginning at page 20, line 1, with the following:

~~TABLE 1~~

50
~~V*SHR PCSR* LLCSC DTLRS GWERA FHDGD TLPWI GSQTA FRVTA MEEPQ~~
100
~~SDPSV EPPLS QETFS DLWKL LPENN VLSPL PSQAM DDLML SPDDI EQWFT~~
150
~~EDPGP DEAPR MPEAA PPVAP APAAP TPAAP APAPS WPLSS SVPSQ KTYQG~~
200
~~SYGFR LGFLH SGTAK SVTCT YSPAL NKMFC QLAKT CPVQL WVDST PPPGT~~
250
~~RVRAM AIYKQ SQHMT EVVRR CPHHE RCSDS DGLAP PQHLI RVEGN LRVEY~~
300
~~LDDRN TFRHS VVVPY EPPEV GSDCT TIHYN YMCNS SCMGG MNRRP ILTII~~
350
~~TLEDS SCNLL GRNSF EVRVC ACPGR DRRTE EENLR KKCEP HHLEP PGSTK~~
400
~~RALPN NTSSS PQPKK KPLDG EYFTL QIRGR ERFEM FRELN EALEL KDAQA~~

~~GKEPG GSRAH SSKLK SKKGQ STSRH KKLME KTEGP DSD*~~

*=Stop codon

TABLE 1

	<u>50</u>
<u>MEEPQ SDPSV EPPLS QETFS DLWKL LPENN VLSPL PSQAM DDLML SPDDI</u>	
	<u>100</u>
<u>EQWFT EDPGP DEAPR MPEAA PPVAP APAAP TPAAP APAPS WPLSS SVPSQ</u>	
	<u>150</u>
<u>KTYQG SYGFR LGFLH SGTAK SVTCT YSPAL NKMFC QLAKT CPVQL WVDST</u>	
	<u>200</u>
<u>PPPGT RVRAM AIYKQ SQHMT EVVRR CPHHE RCSDS DGLAP PQHLI RVEGN</u>	
	<u>250</u>
<u>LRVEY LDDRN TFRHS VVVPY EPPEV GSDCT TIHYN YMCNS SCMGG MNRRP</u>	
	<u>300</u>
<u>LDDRN TFRHS VVVPY EPPEV GSDCT TIHYN YMCNS SCMGG MNRRP ILTII</u>	
	<u>350</u>
<u>ILTII TLEDS SGNLL GRNSF EVRVC ACPGR DRRTE EENLR KKGEF HHELP</u>	
	<u>400</u>
<u>PGSTK RALPN NTSSS PQPKK KPLDG EYFTL QIRGR ERFEM FRELN EALEL</u>	
<u>KDAQA GKEPG GSRAH SSHLK SKKGQ STSRH KKLMF KTEGP DSD*</u>	

* = Stop codon--

Please replace the paragraph beginning at page 32, line 30, with the following:

--To construct the Ad5/p53 viruses, a 1.4 kb HindIII-SmaI fragment containing the full length cDNA for p53 (~~Table 1~~) (Table 1; SEQ ID NO:9) was isolated from pGEM1-p53-B-T (kindly supplied by Dr. Wen Hwa Lee) and inserted into the multiple cloning site of the expression vector pSP72 (Promega) using standard cloning procedures (Sambrook *et al.* (1989)). The p53 insert was recovered from this vector following digestion with XhoI-BglII

and gel electrophoresis. The p53 coding sequence was then inserted into either pNL3C or pNL3CMV adenovirus gene transfer vectors (kindly provided by Dr. Robert Schneider) which contain the Ad5 5' inverted terminal repeat and viral packaging signals and the E1a enhancer upstream of either the Ad2 major late promoter (MLP) or the human cytomegalovirus immediate early gene promoter (CMV), followed by the tripartite leader ~~CDNA~~ cDNA and ~~Ad-5~~ Ad5 sequence 3325-5525 bp in a PML2 background. These new constructs replace the E1 region (bp 360-3325) of Ad5 with p53 driven by either the Ad2 MLP (A/M/53) or the human CMV promoter (A/C/53), both followed by the tripartite leader ~~CDNA~~ cDNA (see Figure 4). The p53 inserts use the remaining downstream E1b polyadenylation site. Additional MLP and CMV driven p53 recombinants (A/M/N/53, A/C/N/53) were generated which had a further 705 nucleotide deletion of ~~Ad-5~~ Ad5 sequence to remove the protein IX (PIX) coding region. As a control, a recombinant adenovirus was generated from the parental PNL3C plasmid without a p53 insert (A/M). A second control consisted of a recombinant adenovirus encoding the beta-galactosidase gene under the control of the CMV promoter (A/C/ β -gal). The plasmids were linearized with either Nru I or Eco RI and co-transfected with the large fragment of [[a]] Cla I digested ~~Ad-5~~ Ad5 d1309 or d1327 mutants (Jones and Shenk (1979)) using a Ca/PO₄ transfection kit (Stratagene). Viral plaques were isolated and recombinants identified by both restriction digest analysis and PCR using recombinant specific primers against the tripartite leader ~~CDNA~~ cDNA sequence with downstream p53 ~~CDNA~~ cDNA sequence. Recombinant virus was further purified by limiting dilution, and virus particles were purified and titered by standard methods (Graham and van der Erb (1973); Graham and Prevec (1991)).--

Please replace the paragraph beginning at page 37, line 10, with the following:

--To determine if p53 recombinant adenoviruses expressed p53 protein, tumor cell lines which do not express endogenous p53 protein were infected. The human tumor cell lines Saos-2 (osteosarcoma) and Hep 3B (hepatocellular carcinoma) were infected for 24 hours with the p53 recombinant adenoviruses A/M/53 or A/C/53 at MOIs ranging 0.1 to 200 pfu/cell.

Western analysis of lysates prepared from infected cells demonstrated a dose-dependent p53 protein expression in both cell types (Figure 5). Both cell lines expressed higher levels of p53 protein following infection with A/C/53 than with A/M/53 (Figure 3; SEQ ID NOS:7 and 8). No p53 protein was detected in non-infected cells. Levels of endogenous wild-type p53 are normally quite low, and nearly undetectable by Western analysis of cell extracts (Bartek *et al.* (1991)). It is clear however that wild-type p53 protein levels are easily detectable after infection with either A/M/53 or A/C/53 at the lower MOIs (Figure 5), suggesting that even low doses of p53 recombinant adenoviruses can produce potentially efficacious levels of p53.--

Please replace the paragraph beginning at page 48, line 22, with the following:

--Adenoviral Vector AANTK: The α -fetoprotein promoter (AFP-P) and enhancer (AFP-E) were cloned from a human genomic DNA (Clontech) using PCR amplification with primers containing restriction sites at their ends. The primers used to isolate the 210 bp AFP-E contained a Nhe I restriction site on the 5' primer and an Xba I, Xho I, Kpn I linker on the 3' primer. The 5' primer sequence was 5'-CGC GCT AGC TCT GCC CCA AAG AGC T-3' (SEQ ID NO:3). The 5' primer sequence was 5'-CGC GGT ACC CTC GAG TCT AGA TAT TGC CAG TGG TGG AAG-3' (SEQ ID NO:4). The primers used to isolate the 1763 bp AFE fragment contained a Not I restriction site on the 5' primer and a Xba I site on the 3' primer. The 5' primer sequence was 5'-CGT GCG GCC GCT GGA GGA CTT TGA GGA TGT CTG TC-3' (SEQ ID NO:5). The 3' primer sequence was 5'-CGC TCT AGA GAG ACC AGT TAG GAA GTT TTC GCA-3' (SEQ ID NO:6). For PCR amplification, the DNA was denatured at 97° for 7 minutes, followed by 5 cycles of amplification at 97°, 1 minute, 53°, 1 minute, 72°, 2 minutes, and a final 72°, 10 minute extension. The amplified AFE was digested with Not I and Xba I and inserted into the Not I, Xba I sites of a plasmid vector (pA/ITR/B) containing adenovirus type 5 sequences 1-350 and 3330 - 5790 separated by a polylinker containing Not I, Xho I, Xba I, Hind III, Kpn I, Bam HI, Nco I, Sma I, and Bgl II sites. The amplified AFP-E was digested with Nhe I and Kpn I and inserted into the AFP-E containing construct described above which had been

digested with Xba I and Kpn I. This new construct was then further digested with Xba I and NgoMI to remove adenoviral sequences 3330 - 5780, which were subsequently replaced with an Xba I, NgoMI restriction fragment of plasmid pACN containing nucleotides 4021 - 10457 of adenovirus type 2 to construct the plasmid pAAN containing both the α -fetoprotein enhancer and promoter. This construct was then digested with Eco RI and Xba I to isolate a 2.3 kb fragment containing the Ad5 inverted terminal repeat, the AFP-E and the AFP-P which was subsequently ligated with the 8.55 kb fragment of Eco RI, Xba I digested pACNTK described above to generate pAANTK where the TK gene is driven by the α -fetoprotein enhancer and promoter in an adenovirus background. This plasmid was then linearized with Eco RI and cotransfected with the large fragment of Cla I digested ALBGL as above and recombinants, designated AANTK, were isolated and purified as described above.--

Please replace the paragraph beginning at page 52, line 29, with the following:

--The efficacy of ACNTK and AANTK for the treatment of HCC was assessed using a ^3H -thymidine incorporation assay to measure the effect of the combination of HSV-TK expression and ganciclovir treatment upon cellular proliferation. The cell lines were infected with either ACNTK or AANTK or the control virus ACN (Wills et al., 1994 supra), which does not direct expression of HSV-TK, and then treated with increasing concentrations of ganciclovir. The effect of this treatment was assessed as a function of increasing concentrations of ganciclovir, and the concentration of ganciclovir required to inhibit ^3H -thymidine incorporated by 50% was determined (ED_{50}). Additionally, a relative measure of adenovirus - mediated gene transfer and expression of each cell line was determined using a control virus which directs expression of the marker gene beta-galactosidase. The data presented in Figure 14 and ~~Table 1~~ Table 2 below show that the ACNTK virus/ganciclovir combination treatment was capable of inhibiting cellular proliferation in all cell lines examined as compared with the control adenovirus ACN in combination with ganciclovir. In contrast, the AANTK viral vector was only effective in those HCC cell lines which have been demonstrated to express α -fetoprotein. In

addition, the AANTK/GCV combination was more effective when the cells were plated at high densities.

~~TABLE 1~~ TABLE 2

<u>Cell Line</u>	aFP	β -gal Expression	ACN	ED50 ACNTK	AANTK
MDAMB468	-	+++	>100	2	>100
BT549	-	+++	>100	<0.3	>100
HLF	-	+++	>100	0.8	>100
CHANG	-	+++	>100	22	>100
HEP-3B	-	+	80	8	8
HEP-G2 LOW	+	++	90	2	35
HEP-G2 HIGH	+	++	89	0.5	4

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 12, at the end of the application.